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Antimycotic activity of phenoxythiazolchloralum

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Abstract

Background: The therapeutic options in invasive candidiasis and aspergillosis are limited and don't provide expected results. Introducing a new drug in the therapeutic practice can improve the quality of life of immunocompromised patients. The aim of this research is to study the antimycotic activity of new substance phenoxithiazolchloralum (MF-0010) on *Aspergillus* spp., *Candida albicans*, and *Saccharomyces cerevisiae.*
Material and methods: Phenoxythiazolchoralum has been kindly offered by the Institute of Chemistry. The standards were offered by *Nicolae Testemitanu* State University of Medicine and Pharmacy. Antifungal activity of the phenoxythiazolchloralum against *Aspergillus* spp. was evaluated by microdilution method. The successive double dilution method was used for determination of *in vitro* susceptibility against *Candida albicans*, and *Saccharomyces cerevisiae.*
Results: For the first time, it was studied *in vitro* susceptibility of MF-0010 against *Aspergillus* spp., *Candida albicans, Saccharomyces cerevisiae*. With at least 0.05 μM/ml difference of MF-0010 MIC value from standards, it can be considered quite more potent than ketoconazole and bifonazole against *Aspergillus fumigatus, Aspergillus versicolor, Aspergillus ochramensis.* The MIC/MCF ratios of MF-0010 are lower than nistatine ones with 0.08 μMol for both pathogens: *Candida albicans*, and *Saccharomyces cerevisiae.*
Conclusions: All analyzed pathogens were susceptible to MF-0010. According to experimental data on *Aspergillus* spp., the antimycotic activity of MF-0010 is quite better than the standards one. The MIC/MCF ratios showed *in vitro* significant susceptibility of MF-0010 against *Candida albicans.*
Key words: Phenoxythazolchloralum, *Aspergillus* spp., *Candida albicans, Saccharomyces cerevisiae*, drug discovery.

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Introduction

Patients with AIDS, organ transplants, cancer, diabetes, autoimmune disorders, biomedical-assist devices, or long-term antibiotic therapy are at major risk of developing mycosis [1, 2]. Reports of invasive aspergillosis and candidiasis in immunocompromised patients have been increasing [3, 4]. *Candida* spp. can affect almost all organ systems in the human body. The genome plasticity and high reproductive capacity constitute a serious risk to human health [5, 6]. Nistatine in one of the few topical drugs for the treatment of cutaneous mycosis caused by this pathogen [7]. Reports of resistance to antimycotic agents have been reported for several years [8]. Thus, every year it is more difficult to predict clinical success /failure and duration of antimycotic therapy. The therapeutic options are quite limited and don't provide expected results [9, 10]. The introduction of new drugs could help to manage this critical situation.

The computer-aided study of novel 5-aryl-2thio-1,3,4-oxadiazoles reported *in vitro* anti-tubercular activity of phenoxythiazolchloralum (MF-0010) (fig. 1) against *Mycobacterium tuberculosis* $H_{37}Rv$ [11]. Pharmacophore groups –NH-, =C=O, =N-N=, -Cl produce three-dimensional arrangements that are required for anti-tubercular activity [12].

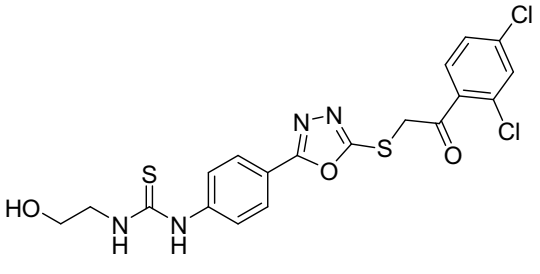

Fig. 1. The chemical structure of phenoxythiazolchloralum (MF-0010)

Acute toxicity tests reveal that MF-0010 in mice after intraperitoneal injection and intragastric administration for a single dose suggests that compound has minimal toxicity, and can be placed in the toxicity class 5 according to TG 423 Acute Toxic Class Method (OECD) [13]. Organoleptic characteristics, solubility, melting temperature and UV absorption spectra are described by O. Butescu [14]. It was observed that compound has poor solubility in polar solvents (water, methanol, ethanol, acetonitrile, acetone) and low bioavailability that limits the use as antitubercular agent. Following research of chemical structure showed that substance could have other biological activities. The

presence of the azole ring (pharmacophore group of azole antimycotic drug) suggests that the studied substance may have antimycotic activity.

The aim of this research is to study the antimycotic activity of MF-0010 on *Aspergillus* spp., *Candida albicans*, and *Saccharomyces cerevisiae.*

Material and methods

***Aspergillus* spp.**

For evaluating of the antifungal activity of the phenoxythiazolchloralum compound against *Aspergillus* spp. was used the microdilution method described by E. Stingaci et al. [15].

Candida albicans and Saccharomyces cerevisiae

For evaluating of the antifungal activity for *Candida albicans*, and *Saccharomyces cerevisiae* was used the successive double dilution method. For this, at the initial stage, 1 mL of Sabouraud broth for test fungus was introduced into a series of 10 tubes. Subsequently, 1 mL of the analyzed compound was dropped into the first test tube.

Then, the obtained mixture was pipetted, after that 1 mL of it was transferred to the next tube, so the procedure was repeated until the tube No 10 of the series. Thus, the concentration of the initial preparation decreased 2-fold in each subsequent tube.

At the same time, 24 hour test fungi of *Candida albicans* and *Saccharomyces cerevisiae* were prepared. Initially, suspensions of test fungi were prepared with optical densities (D.O.) of 0.5 according to the McFarland index. Subsequently, 1 mL of the obtained fungal suspension was dropped in a tube containing 9 mL of sterile distilled water. The content of the tube was mixed, after which 1 mL was transferred to the tube No 2 of the 5-tube series containing 9 mL of sterile distilled water.

From the 5-th tube of the series was taken 0.1 mL of the fungal suspension, which represents the seeded dose and added to each tube with titrated preparation. Subsequently, the tubes with titrated preparation and the seeded doses of the microorganisms were put in the thermostat at 35°C for 24 hours. On the second day, a preliminary analysis of the results was made. The last tube from the series in which no visible growth of microorganisms has been detected was considered to be the minimal inhibitory concentration (MIC) of the preparation.

For the estimation of the minimum fungicidal concentration (MFC), the contents of the test tubes with MIC and with higher concentrations were seeded on Sabouraud agar from Petri dishes with the use of the bacteriological loop. The seeded dishes were kept in the thermostat at 35°C for 24 hours. The concentration of preparation, which did not allow the growth of any colony of microorganisms, was considered to be the minimal fungicidal concentration of the compound [16].

Results

***Aspergillus* spp.**

For the first time, it was studied *in vitro* susceptibility of MF-0010 against *A. fumigatus, A. versicolor, A. ochramensis* and *A. niger.* The MIC and MFC values of MF-0010 against *Aspergillus* spp. ranged from 0.23 μM/ml – 0.62 μM/ml (fig. 2) and 0.62 μM/ml –1.24 μM/ml (fig. 3), respectively. The highest values of MIC and MCF of MF-0010 are related to *A. niger*. Thus, the use of MF-0010 against this pathogen is not appropriate.

The MIC values of MF-0010 on *A. fumigatus, A. versicolor, A. ochramensis* and *A. niger* are 0.23 μM/ml -0.31 μM/ml, which are lower than MICs of standards: ketoconazole (0.28 μM/ml-0,38 μM/ml) and bifonazole (0.32 μM/ml-0.48 μM/ml). With at least 0.05 μM/ml difference from standards, MF-0010 can be considered quite more potent than ketoconazole and bifonazole.

The MCF value of MF-0010 against *A. fumigatus, A. versicolor, A. ochramensis* and *A. niger* is 0.62 μM/ml, which is lower than MFC values of standards: ketoconazole – 0.94 μM/ml (exception is *A.ochramensis* – 0.38 μM/ml) and bifonazole – 0.64 μM/ml. With a 0.02 μM/ml difference from standards, MF-0010 has the same antimycotic activity as bifonazole, and better one than ketoconazole.

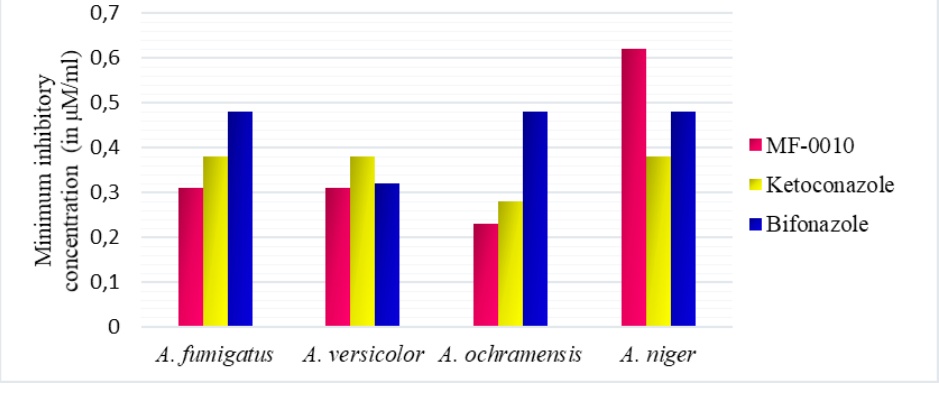

Fig. 2. The Minimum Inhibitory Concentration (MIC) of MF-0010, ketoconazole, bifonazole against *A. fumigatus, A. versicolor, A. ochramensis* and *A. niger*

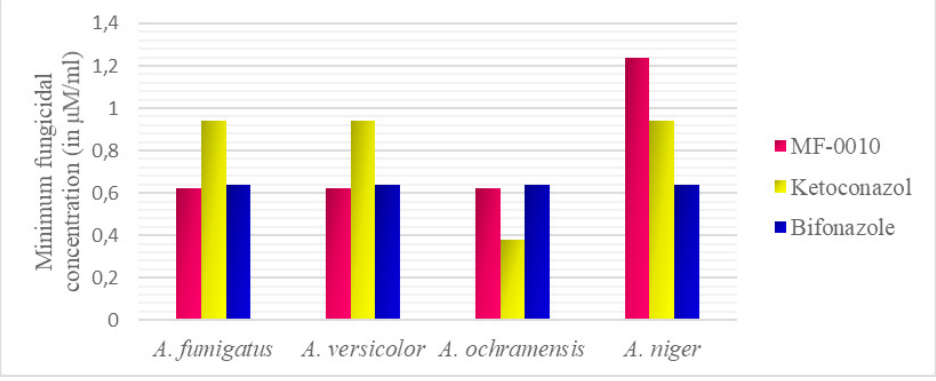

Fig. 3. The Minimum Fungicidal Concentration (MCF) of MF-0010, ketoconazole, bifonazole against *A. fumigatus, A. versicolor, A. ochramensis* and *A. niger*

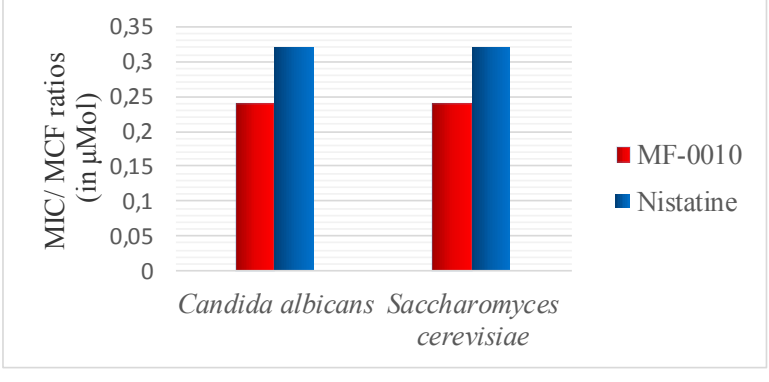

Fig. 4. MIC/MCF ratios of MF-0010, nistatine against *Candida albicans, Saccharomyces cerevisiae*

Candida albicans* and *Saccharomyces cerevisiae

For the first time, it was studied the *in vitro* inhibition potential of MF-0010 against *Candida albicans, Saccharomyces cerevisiae* (fig. 4).

The MIC/MCF ratios of MF-0010 for inhibition of *Candida albicans, Saccharomyces cerevisiae* are lower than nistatine ones with 0.08 µMol for both pathogens. Thus, we can conclude that MF-0010 is more potent active molecule than nistatine against *Candida albicans*. Further research is needed to create topical forms and confirm by clinical trial the pharmacological action, pharmacokinetic profile and safety of formulations with MF-0010. Several types of topical medication including creams, ointments, and pastes can be designed.

Conclusions

In this study, we found that all analyzed pathogens were susceptible to MF-0010. According to the experimental data, the antimycotic activity of MF-0010 is quite better than the standard one. The MIC and MCF values of MF-0010 show a good potency against *Candida albicans*, and new studies are warranted in order to design optimized formulations, to analyze *in vivo* the efficacy and quality assurance of formulations.

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Authors' contribution
AP interpreted the data, drafted the first manuscript. VV formulated the research hypothesis, revised the manuscript. SP synthetized MF-0010, performed the technological part of laboratory work. LL designed the study, conducted the laboratory work. AU performed the technological part of laboratory work. FM interpreted the data, revised the manuscript. All the authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate
No approval was required for this study.

Conflict of Interests
No competing interests were disclosed.